

CORTISOL AS AN INDICATOR OF HYPOTHALMIC-PITUITARY-ADRENAL AXIS DYSREGULATION IN PATIENTS WITH PANIC DISORDER: A LITERATURE REVIEW

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SUMMARY

Dysregulation of hypothalamic–pituitary–adrenal axis (HPA) is seen in numerous mental disorders. Data of HPA axis disturbance in panic disorder are inconsistent. In panic disorder HPA axis hyperactivity has been observed with elevated cortisol levels. However, hypocortisolism has also been noted. Salivary cortisol as a biomarker of HPA-axis activity has received special attention. The aim of this paper is to review the findings on cortisol levels in panic disorder.

Key words: hypothalamic–pituitary–adrenal axis - salivary cortisol - cortisol awakening response - panic disorder

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INTRODUCTION

Panic disorder is a persistent anxiety disorder leading to significant disability and a high number of hospital admissions. The efficacy of pharmacological treatment in panic disorder is limited and novel treatments that are based on current understanding of conventional neurotransmitter function are unlikely to be more effective or better tolerated than the current treatment (Elnazer 2014).

Although the pathophysiological mechanisms underlying panic disorder is not well identified to date, the biological hypotheses has been postulated. The neuroanatomical hypothesis of Gorman described the "fear network" in the brain which is centred in amygdala and involves its interactions with the hippocampus and medial prefrontal cortex. It is speculated that a similar network is involved in panic disorder. According to Gorman, patients with panic attacks inherit a particularly sensitive central nervous system fear mechanism and both heritable factors and stressful life events are responsible for the onset of panic disorder (Gorman 2000). Some data indicate the decrease of receptor 5-HT_{1A} sensitivity in different areas of the fear neuronal network and decrease ability to serotonin bindings in patients with panic disorder (Nash 2008). Due to massive stress experienced by patients with panic disorder a strong activation of the HPA axis can be expected in those patients. However studies that have measured cortisol during natural panic attacks have found either no or a little increase of "stress hormone" in patients with panic disorder (Sigmund 2011). Researchers have not demonstrated a consistent pattern of endocrine disturbances in panic disorder.

CORTISOL LEVELS AS A MEASURE OF HYPOTHALMIC-PITUITARY- ADRENAL AXIS ACTIVITY

The HPA axis is a neuroendocrine system involved in adaptation to change and challenge. Functioning of the HPA axis is a subject of psychiatric research and both hypo- and hyperactivity of HPA-axis, measured in cortisol level, have been found in different psychiatric disorders (Staufenbiel 2013). The most characteristic stress response is the release of the adrenocorticotropic hormone (ACTH) and cortisol into the blood stream as a result of activation of the HPA axis. In addition to the HPA axis, acute stress also activates the sympathetic division of the neurovegetative nervous system as part of the fight/flight reaction (Graeff 2010).

The HPA axis dysfunction in mental disorders may not be a consequence of these disorders per se but the manifestation of persistent neurobiological abnormalities that predispose to their development dependant on specific combinations of stress exposure (Elnazer 2014). The ongoing disruption of HPA axis towards hyper- or hypoactivity may have adverse impacts on mental and physical wellbeing (Elnazer 2014).

The HPA axis exposed to chronic stress shows both habituation and facilitation. When the same stressor is delivered repeatedly the diminution of glucocorticoid responses to the stimulus is observed. Facilitation is present when subjects repeatedly exposed to one stimulus are presented with novel stressor-in such a situation a large rise in glucocorticoids is observed (Herman 2005).

It is not clear whether the HPA axis is involved in pathophysiology of panic disorder. Several studies using

different investigational methods were performed in patients presenting different severity of illness, diverse times, periods of the day; total and free cortisol in plasma, saliva and urine cortisol was measured which led to inconclusive and incomparable results (Erhardt 2006). The research confirmed the hypothesis that HPA axis regulation is impaired in anxiety and depression patients, however different biological mechanisms of the HPA axis dysregulation is present in both of these disorders. In panic disorder the brain structures are important (amygdala, hippocampus, prefrontal cortex) involved in hyperarousal, perception of external and internal stimuli and cognitive assessment (Table 1).

Table 1. Summary of cortisol levels in panic disorder

| Author | Basal cortisol levels |
|-------------------------|---|
| Plasma cortisol | |
| Nesse et al. 1984 | |
| Roy-Byrne et al. 1986 | Significantly elevated |
| Goldstein et al. 1987 | |
| Hollander et al. 1989 | Elevated nocturnal |
| Abelson and Curtis 1996 | cortisol levels |
| Liebowitz et al. 1985 | |
| Villacres et al. 1987 | |
| Cameron et al. 1987 | |
| Stein and Uhde 1988 | |
| Woods et al. 1988 | Not elevated |
| Gurguis et al. 1991 | |
| Brambilla et al. 1992 | |
| Brambilla et al. 1995 | |
| Siegmund et al. 2011 | |
| Urinary free cortisol | |
| Bandelow et al. 1997 | |
| Bandelow et al. 2000b | Elevated |
| Wedekind et al. 2000 | |
| Lopez et al. 1990 | Elevated only in patients with agoraphobia and secondary depression |
| Uhde et al. 1988 | Not elevated |
| Salivary cortisol | |
| Bandelow et al. 2000a | Elevated (correlated to symptom severity) |
| Vreeburg et al. 2010 | |
| Vreeburg et al. 2013 | Elevated |
| Wedekind et al. 1999 | |

CORTISOL MEASUREMENT

Cortisol is a glucocorticoid hormone synthesized from cholesterol in the adrenal cortex. Cortisol is commonly known as "stress hormone" being released in high doses under stressful conditions. Its synthesis is stimulated by ACTH being released by CRH (corticotrophin releasing hormone) from the hypothalamus and is inhibited by cortisol (negative feedback). In human circulation, approximately 75% of cortisol is protein-bound (CBG-cortisol binding protein). Cortisol affects

the coordination of brain and body functions involved with coping with the stressor (Staufenbiel 2013). Cortisol has a circadian rhythm of excretion and time of the assessment is expected to be an important factor in its measurement. Low values of cortisol are present at awakening, followed by peak values 30min. after awakening and a steady decline during the rest of the day (Meewisse 2007). Glucocorticoid release from the adrenal cortex is modulated in part by the hypothalamus and the anterior pituitary, which secretes stimulating hormones (e.g. corticotrophin-releasing hormone and adrenocorticotrophic hormone, respectively) when an energetic need is perceived and decreased production of stimulating hormones when energetic needs are perceived as stable (Sapolsky 1992). The timing of the cortisol test is very important because of the way cortisol levels vary throughout a day.

There are a few ways to measure HPA-axis functions: CAR (cortisol awakening response) is a measure of the dynamics of the cortisol awaking response emphasising changes over time after awaking (samples are collected: on awakening, at 30min., at 45min., and 60min.), evening cortisol (measure at 22.00; 23.00; evening cortisol is definite as an average of the two evening cortisol levels), baseline plasma cortisol, urinary cortisol, hair cortisol, cortisol suppression by dexamethasone-DST (dexamethasone suppression test), DEX-CRH test. The cortisol levels obtained by aforementioned techniques show considerable intra and inter individual differences due to cortisol's circadian rhythm and its pulsatile secretion (Sapolsky 1992). The highest levels of cortisol are present shortly after awakening, typically decreasing values during the day, with the lowest levels around the midnight (Jezova 1996). Cortisol levels rise sharply (50-160% in saliva) during the first 30 to 40 minutes after wakeup, returning to the awakening baseline within 60 to 75 minutes, and declining more gradually thereafter.

Salivary cortisol measures are used to assess basal HPA-axis activity (Holleman 2012). Saliva sampling is a stress-free, non-invasive, easy to perform, useful method for studying neuroendocrine abnormalities in psychiatric disorder. Patients may be examined not in the laboratory, but in natural conditions. For hygienic collection of saliva samples Salivette swabs are used, then samples are kept frozen -20°C until assay. The Salivette sampling device consists of cotton swabs which the patients chew for 2 minutes and then they are transferred to the plastic tube of the device. The patient is instructed to refrain from eating, smoking, drinking tea or coffee, or brushing teeth 15 min to sampling and no dental work is allowed in the 24 hours preceding sample collection. Salivary cortisol levels are relatively insensitive to repeated thawing and refreezing (Kirschbaum 1993). Saliva samples can be stored at room temperature or in the participants' home refrigerator or freezer until they are delivered to the lab. Cortisol levels measured in saliva correlate highly with

free cortisol in blood. Due to a partial conversion of cortisol to cortisone during passage through the salivary glands, the absolute level of free cortisol in saliva is 10% to 35% lower than it is in blood. Correlations with total blood concentrations (bound and free fractions) are also high, but the slope of the regression line becomes steeper at higher cortisol concentrations, after CBG-binding sites in blood are fully occupied. CBG levels can vary both within and between individuals, for example during pregnancy or with oral contraceptive use. Movement of cortisol from blood to saliva occurs by passive diffusion, so that salivary levels are independent of the flow rate of saliva. Changes in plasma and salivary cortisol levels are closely correlated. After injection of cortisol, salivary levels increased within 1 minute and peak concentrations in blood are seen 2 to 3 minutes later in saliva. The proportion of salivary cortisol to total cortisol is about 1-2% in the lower range, and about 8-9% in the upper range. Salivary cortisol levels have to be treated with caution, since they behave in non-linear pattern to serum levels in response to a challenge or under conditions which affect CBG levels, such as oral contraceptives, menstrual cycle or pregnancy. The salivary cortisol assay showed such aberrations already for cortisol levels above 10 nmol/l (Vining 1986). This is an important issue, which is often neglected in psychobiological research. However, the relation between total and free cortisol levels in blood changes once CBG is saturated (Kirschbaum 1993).

The CAR reflects the natural response of the HPA-axis on awakening being the acute rise in hormone level after awaking. It is under genetic control and exhibits greater intra-personal stability than single morning cortisol measurements and is secreted in circadian pattern. The CAR appears to be moderately stable within single subjects, from day to day and over longer periods of several weeks to months, but it can vary in relation to short-term influences, such as the stressfulness of a workday compared to a weekend. It is considered to be regulated in anticipation of demands of the upcoming day and more reliable measure for the acute reactivity of the HPA axis (Holleman 2012). Some 90% of cortisol is bound to CBG (corticoid-binding globulin). Only the free fraction of cortisol is biologically active (Kirschbaum 1993, Hellhammer 2009). Salivary cortisol reflects free, unbound cortisol plasma levels (Bandelow 2000) and is thought to be a promising biomarker of stress system activity. A researcher must be aware of variables such oestrogens (gender, menstrual cycle, oral contraceptives, or medical conditions can affect cortisol CBG binding and HPA-axis (Hellhammer 2009). It has been suggested that CAR is under regulatory control of hippocampus. A reduced hippocampus has been associated with the blunted CAR. It is possible that a lower CAR among depression and anxiety disorders is associated with unfavourable course of these disorders and may be indicative of underlying exhaustion of the HPA axis. A

higher cortisol awakening curve is associated with the onset of depressive or anxiety disorders (Vreeburg 2013). Salivary and blood cortisol are highly correlated with each other (Petrowski 2012). Under baseline (resting and nonstressed) conditions, plasma glucocorticoid levels vary predictably across a 24-h period (circadian variation) and, in some species, across the year in a seasonal pattern (Romero 2002).

DST (dexamethasone suppression test) provides information on the negative feedback system of the HPA axis, since dexamethasone reduces cortisol level by acting on the pituitary (Vreeburg 2013). Administration of 1mg of DEX to normal subjects suppresses the nocturnal surge in ACTH production and, as a consequence, cortisol levels are low when measured the next morning (Huizenga 1998). There is evidence that increased cortisol responses to the DST predict relapse in remitted outpatients with depressive disorder or panic disorder. The DEX-CRH test: administration of 1.5mg of DEH orally, the next day a single injection of 100microgram CRH intravenously. Then the HPA axis reaction can be monitor by use parameters: plasma cortisol and ACTH before the CRH administration (reflects the suppression effect of dexamethasone pre-treatment); plasma cortisol and ACTH after CRH injection-reflects the stimulating effects of CRH injection on HPA system (Erhardt 2006).

The use of hair in cortisol assays has been conducted since 2004. The extraction of cortisol from human hair gives unique possibility to show the average long-term activity of the HPA-axis before and after stressful event. It is a non-invasive technique and it is not affected by oral contraceptives. Hair cortisol probably reflects the amount of free, unbound cortisol. It is usually cut from the scalp. As hair grows about one centimetre per month it is possible to assess the long-term total cortisol exposure (months to years). This technique gives a possibility to assess a cortisol level before the event-whether it concerns the onset of a mental disorder. In panic disorder level of hair cortisol was reported to be decreased (Staufenbiel 2013).

Urine analyses may be useful to assess urinary cortisol and its metabolites levels. A 24 hours urine collection should be used to determine urine cortisol level. Urine may be collected into containers with or without boric acid as a preservative. Unidentified substances in the urine may interfere with the extraction of cortisol from urine, producing falsely high values (Yehuda 2003). Renal conditions and hydration status also may affect cortisol level. 70% of the cortisol biologically inactive metabolites are excreted in urine. In urine 1% of free blood cortisol is excreted. Overnight or 24 hours urine measures have the advantage to provide integrative HPA axis measures over larger time periods, but the compliance is poor (complete urine collection is necessary). It is said that urinary cortisol is valid to approach if not only cortisol but also cortisol metabolites are assessed as HPA axis measure

(Bandelow 2000). Studies compared averaged cortisol values from multiple day time salivary collections to 24-hours urinary cortisol reported little or no association between salivary and urinary indices. It is probably due to the fact diurnal changes in urinary cortisol show a lag of approximately 4 hours relative to salivary and plasma cortisol (Yehuda 2003).

HPA AXIS ABNORMALITIES IN PATIENT WITH PANIC DISORDER

Until the sixties of the twentieth century it was considered that glucocorticoids act mainly in the peripheral nervous system. In 1968 McEwen discovered GR receptors (glucocorticoid receptors) in rat's hippocampus. It was the beginning of data which were looking for cortisol action on the course and extinction of stress reaction in the central nervous system (McEwen 2007). GR and MR receptors have different localization and affinity to glucocorticoids. While MR occur mainly in the limbic system (hippocampus, amygdala) GR is widespread in the brain but its expression is especially high in the regions engaged in mechanisms of stress (hypothalamus, hippocampus, amygdala, nuclei of brain stem and pituitary gland) (Trapp 1994, Ratka 1989). In hippocampus MR and GR occur in the highest concentration (Dallman 1993). Corticosteroids regulate HPA axis in the reverse feedback mechanism. That process is important for the body homeostasis (Uhde 1988). In physiological conditions inhibitory action of low concentration of corticosteroids on HPA axis in circadian rhythm MR occurs. MR inhibits production and secretion of CRH (corticotrophin releasing hormone) in hypothalamus (Uhde 1988, Lopez 1990).

In a few studies elevated baseline plasma cortisol levels were reported in PD patients (Nesse 1984, Roy-Byrne 1986, Goldstein 1987). In several others researches cortisol plasma levels were not confirmed being elevated (for review: Bandelow 2000). Urinary free cortisol levels in PD was normal (Woods 1988) or elevated in subjects with complicated PD (comorbidity with depression, agoraphobia) (Coplan 1998). Urinary cortisol was also elevated in Bandelow's study (Bandelow 2000) being only measured during the night. Nocturnal awakenings in the laboratory may trigger cortisol release and elevation (Erhardt 2006). According to Bandelow (1997) and earlier Abelson and Curtis (1996) findings cortisol elevations seem to be more noticeable during the night. Daytime cortisol levels were similar to control. In Bandelow's study (1997) salivary and urinary cortisol levels were highly stable over 4 weeks period suggesting that it is a rather constant change. During the day patients and healthy individuals may be confronted with stressful life events and physical activity what may increase cortisol levels (Jezova 1996).

Increased cortisol levels have been reported in healthy subjects under stress provocation (Hollander 1989). In patients with panic disorder provoked by lactate infusion or carbon dioxide inhalation any cortisol increase was found during panic attacks (Woods 1988, Coplan 1998, Cameron 1987). However, in patients who experienced panic attack after lactate infusion a rise in cortisol was reported before infusion (Woods 1988, Shekhar 1999). Data analysing cortisol levels during non-chemically provoking panic attacks are inconsistent. Cortisol elevation was not significant in two studies where blood was sampled (Woods 1988, Shekhar 1999). Also, during unprovoked panic attacks elevated cortisol levels in saliva samplings were demonstrated (Bandelow 2000).

Some data suggest that the HPA axis is disturbed in patients with panic disorder. Some reports do not confirm HPA activation during panic attacks. The reason for the inconsistencies is partly due to methodological differences but also psychological factors (novelty, social separation, lack of control, perceived threat) modulate the system. The contradictory and inconsistent findings on HPA abnormalities in patients suffering from panic disorder. Abelson et al. (2007) concluded that this disorder is characterized by an enhanced sensitivity to novelty with a heightened fear of panic in unfamiliar environments and due to that hypersensitivity panic disorder patients would therefore account for HPA axis abnormalities (Jezova 1996). These HPA axis abnormalities may be a trait that enhances general vulnerability to psychopathology. Hyperactivity of the amygdala, what was proven in anxiety disorders (Shekhar 1999), could also contribute to panic and lead to enhanced HPA axis reactivity (Coplan 1998).

Although panic attacks cause a major sympathetic stimulation they have little effect on the HPA axis (Graeff 2010). In contrast, anticipatory or generalized anxiety activates both the HPA and the sympatho-adrenal axes. The HPA axis abnormalities in some studies may be due to anticipatory anxiety generated by the procedure of collecting salivary samples (Erhardt 2006).

In order to check the hypothesis of HPA axis hyperactivity to novel, threatening, uncontrollable situations in PD patients Petrowski et al. (2010) used TSST (Trier Social Stress Test). Patients were collecting saliva samplings throughout the TSST sessions and in the two weekday mornings CAR). Non-response to psychological stressors was observed during TSST, normal cortisol awaking responses were reported. These data contradict conclusions drawn by Abelson et al. (2007) that PD patients would show an enhanced HPA responsiveness to novel and uncontrollable situations (Petrowski 2010). It is possible that the cortisol non-response to acute panic attacks reflects successful habituation to repeated stimulation by complex emotional events (Petrowski 2012). According to this hypothesis a

pattern of HPA (or cortisol) non-responsiveness to psychosocial stress seems to be rather specific and represents a vulnerability factor for later development of PD (Petrowski 2012).

The Petrowski study (2012) showed the dissociation of the plasma cortisol and plasma ACTH levels in the DEX-CRH test of patients with panic disorder as compared to healthy controls and patients with depression. The patients with PD showed a decreased CRH-induced plasma cortisol response pattern, however no change in ACTH levels were observed in patients with PD and healthy control. Panic disorder patients with duration of the disease longer than 2 years showed remarkably higher HPA-axis reactivity under CRH-injection than patients suffering from PD for two years or less. There is high probability that patients with PD with longer time of disease will tend to develop a hyperactivity of HPA. According to Graeff specific neurobiological processes underlie GAD and PD and analysed data suggest that while anxiety activates both the HPA and the sympatho-adrenal axes, the panic attack causes major sympathetic activation, but has little effect on the HPA axis (Graeff 2010). The Petrowski study (2013) provided second evidence for the HPA axis hyporesponsiveness in patients with panic disorder under psychosocial stress. In psychosocial stress stimulation there were no differences between patients and healthy controls in baseline cortisol and baseline ACTH levels measured in blood and saliva. They suggest that patients with panic disorder develop hypo-responsiveness over time. The decrease of the HPA axis activity may be a compensatory strategy. It may be a result of a down regulation of the pituitary receptors for the CRH (corticotrophin-releasing hormone) or diminished adrenocortical sensitivity to ACTH (Petrowski 2013). During flooding therapy the dissociation of fear and stress hormone levels was observed. In spite of increased fear in phobic situation no significant cortisol and ACTH levels were presented (Siegmond 2011). Patients with panic disorder do not activate cortisol at the time when it should occur. The hippocampus is the special place where interaction between corticosteroids and serotonin system take place. In healthy animals, after stress exposure or after corticosteroids infusion decrease of serotonin neurotransmission, decrease of serotonin level and 5-HT_{1A} receptors in brain are present (De Kloet 2005). Hypercortisolemia seems to be responsible for the decrease of 5HT_{1A} neurotransmission.

CONCLUSION

The aetiology of panic disorder is multifactorial including environmental, genetic, and neurobiological factors (Huizenga 1998). Associations between endocrine function and mental health have been explored for years. The HPA axis plays role in pathogenesis of anxiety disorders. The HPA axis modulates neurotransmission and influences memory (Lopez 1990).

Surprisingly there is not a sudden rise and HPA axis activation during a panic attack, but rather subtle alterations in HPA axis activity which may correlate with severity of anticipatory anxiety, avoidance and general illness severity (Nash 2008). It is not clear whether the dysfunctions of the HPA axis are a potential cause of panic disorder or a consequence of permanent stress induced by recurrent panic attacks (Bandelow 2013).

It is unclear whether HPA axis dysregulation predicts the course of anxiety disorders. There is evidence that increased cortisol responses to the DST predict relapse in remitted patients with panic disorder. But there is no evidence that baseline cortisol levels or cortisol responses on the DST are related to the treatment response or outcome of panic disorder (Bandelow 2013).

A lower cortisol awaking response seems to predict an unfavourable, chronic course of anxiety disorders and may be indicator of underlying exhaustion of the HPA axis. A lower CAR is an indicator of hypocortisolism. A possible mechanism for hypocortisolism is down regulation of CRH receptors in the pituitary. As higher CAR was related with the presence of panic disorders with agoraphobia it is possible higher CAR is associated with the onset of panic disorders with agoraphobia.

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